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EVALUATION OF FUEL CELL WATER FOR HUMAN CONSUMPTION

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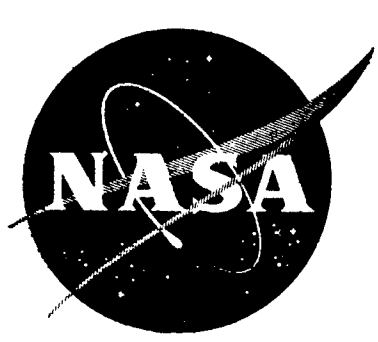

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Foreword

This study was initiated by the Biomedical Laboratory of the Aerospace Medical Research Laboratories, Aerospace Medical Division, Wright-Patterson Air Force Base, Ohio, at the request of Dr. W. W. Kemmerer, of the National Aeronautics and Space Administration, Manned Spacecraft Center, Houston, Texas. The research was conducted by the Aerospace Medical Research Laboratories under the terms of NASA Defense Purchase Request R-85, and by the Miami Valley Hospital Research Department, Dayton, Ohio 45409, under Contract AF 33(657)-11716. Dr. Bernard J. Katchman was principal investigator for the Miami Valley Hospital Research Department, and Dr. Arnold R. Slonim for Aerospace Medical Research Laboratories. Dr. Sheldon London, of the Biospecialties Branch, Physiology Division, was the contract monitor for the Biomedical Laboratory. The work was performed in support of Project 7164, "Biomedical Criteria for Aerospace Flight," Task No. 716410, "Aerospace Sanitation and Personal Hygiene."

The authors would like to express their appreciation to the following individuals who made significant contributions to the study:

Dr. A. Prince, Chief, Biospecialties Branch; Lt. K. J. Smith of Biospecialties Branch; Professor William D. Gray, Southern Illinois University, Carbondale, Illinois; Mr. Robert Kroner, Water Quality Section, USPHS, Cincinnati, Ohio; and to Airman K. M. Offner, Mr. C. M. Cox, and Mr. F. C. Crawley of the Biospecialties Branch.

This technical report has been reviewed and is approved.

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Abstract

Water obtained from a hydrogen-oxygen fuel cell was subjected to chemical, organoleptic, and microbiological analyses and found to be acceptable according to the U. S. Public Health Service Standards. To ascertain the acceptability of this water, eight male subjects were confined to a self-care unit at Miami Valley Hospital, Dayton, Ohio, for a 2-week period and served a controlled 4-meal-per-day diet, low in water content. A minimum of 2 liters per day of either distilled water or fuel cell water was drunk by the subjects as follows: two subjects were served distilled water for 2 weeks, serving as controls; four were served fuel cell water for 2 weeks; and two were served fuel cell water for one week, followed by distilled water for the second week. Twenty-four hour surveillance of the subjects was maintained. They were examined by a physician at least once daily; daily urinary and periodic blood samples were taken for routine laboratory examination. The subjects showed no clinical symptoms at the end of the test period, and the fuel cell water was found to be as acceptable as distilled water in constituting part of a daily diet for the 2-week period.

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SECTION I.

Introduction

In view of the planning for manned space missions of extended duration, it is mandatory to provide an adequate source of potable water for the astronauts. One possible source of drinking water in a spacecraft is that formed by electrochemical reaction that takes place in the fuel cell. However, contaminants contributed by the cell materials or infiltration may show wide variation in quantity and quality depending on the accumulated time of operation and the load placed on the cell as well as on cell design.

An earlier report[1] discusses the results of tests to determine any subacute toxic effect of the fuel cell product water when given to monkeys. That study failed to reveal any apparent subacute oral toxicity of the fuel cell water in monkeys when administered for a 14-day period. It also concluded that product water having similar chemical and physical properties should present no serious hazard to humans when consumed as drinking water for short periods of time. Since the physical and chemical properties of fuel cell water vary with time, it was concluded that it might not be applicable to all situations.

Before actually recommending fuel cell water for use on manned space missions, it was necessary to determine if water from that source would be acceptable for human consumption.

[1] Ziegler, Ralph F., *Effects of Oral Administration of a Fuel Cell Product Water to Macaca Mulatta*, AMRL-TR-65-176 (AD 634 315), Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, March 1966.

SECTION II.
Procedures

WATER EVALUATION

The fuel cell water used in this study was obtained from the General Electric Gemini fuel cell and was furnished by McDonnell Aircraft Corporation. Approximately 283 liters (75 gallons) of fuel cell water were obtained for use in the study. It was contained in 41 plastic carboys of assorted sizes. None of the containers had been opened from the time they were filled until they arrived in the laboratory for evaluation. The carboys were arranged in order of age of the water. The oldest had been filled 27 February 1965 and the most recent, 8 March 1965. The contents of nine of the carboys were sampled aseptically and randomly for microbiological and chemical analyses. In addition, microbiological and chemical analyses were made on a pooled sample, consisting of 10-ml samples from each of the 41 carboys. Chemical analyses indicated that the water was acceptable (appendix I), but the microbiological analyses (appendix II) showed an excessive amount of bacteria and fungi.

Carboys containing the oldest water were eliminated from the experiment. Water from the remaining 32 carboys was pooled and filtered to provide 206 liters for human evaluation of organoleptic acceptability.

SUBJECTS

Eight male subjects were selected and admitted to the Miami Valley Hospital on 15 August 1965 at 2000 hours and received a final briefing at that time. The subjects were between 21 and 34 years of age and had been screened by objective psychological tests, subjective psychological interview, medical history, and physical examination. They were housed in a 4-bed ward and two 2-bed rooms in the self-care unit. At 0700 on 16 August 1965, the subjects were given a pretest physical examination, and pretest blood and urine samples were collected for analyses.

TEST PLAN

The space missions conducted by the National Aeronautics and Space Administration under the Gemini programs were designed for a period of approximately two weeks. The protocol of the present study was based on that requirement.

The psychological and physiological factors involved in consuming the fuel cell water could best be evaluated by giving some of the subjects fuel cell water for the entire experiment, others only distilled water as controls, while giving a third group both distilled and fuel cell water. The latter group would serve as their own controls. The pattern of water consumption is given below:

TIME PERIOD	SUBJECT NUMBER							
	1	2	3	4	5	6	7	8
1st Week (days 1-7)	FCW	FCW	FCW	FCW	FCW	DW	FCW	DW
2d Week (days 8-14)	DW	FCW	FCW	FCW	FCW	DW	DW	DW

FCW = fuel cell water
DW = distilled water

TABLE I.

COMPOSITION OF THE DIETARY REGIMEN*

Meal	*Menu A*	*Menu B*	*Menu C*
Breakfast 0800	Sliced Banana Scrambled Eggs Bacon Toast Butter Jelly	Applesauce Poached Eggs Toast Butter Jelly	Sliced Banana Hard Boiled Eggs Sausage Toast Butter Jelly
Dinner 1200	Roast Beef Baked Potato Green Beans Bread Butter Peaches Brownies	Beef Tenderloin French Fried Potatoes Peas Bread Butter Apricots Cookies	Hamburger/Bun Catsup Fruit Cocktail Pound Cake
Supper 1600	Baked Chicken Noodles Corn Bread Butter Angel Food Cake	Pork Loin Mashed Potatoes Carrots Bread/Butter Canned Pears Graham Crackers	Sliced Turkey Mashed Potatoes Wax Beans Bread/Butter Dark Sweet Cherries Cookies
Night Meal 2000	Ham Sandwich/Butter Sliced Pineapple Sugar Wafers	Turkey Sandwich/Butter Sliced Pineapple Sugar Wafers	Roast Beef Sandwich/Butter Sliced Pineapple Sugar Wafers
Between Meals	Hard Candy	Hard Candy	Hard Candy

*Average Daily Composition: 2536 kcal, Protein – 120.3 g, Fat – 91.6 g, Carbohydrate – 307.6 g
Caloric Distribution, percentage: Protein – 19.0, Fat – 32.5, Carbohydrate – 48.5

At 0800, the experiment was initiated with the consumption of the first meal. During the 2-week test, the subjects were served a controlled 3-day cycle, 4-meal diet, which included either distilled or fuel cell water. Table I shows the dietary regimen. All foods were selected on the basis of low water content to stimulate adequate consumption. The meals were served at 0800, 1200, 1600, and 2000. Between meal snacks of hard candy were served, another means of stimulating water consumption. All foods were served at appropriate temperatures and the water was stored and served at room temperature. All food served each day was consumed with a minimum of 2000 ml distilled or fuel cell water. Each subject was required to perform an organoleptic rating of each meal, according to the following hedonic scale:

9 – Like Extremely
8 – Like Very Much
7 – Like Moderately
6 – Like Slightly
5 – Neither Like Nor Dislike
4 – Dislike Slightly
3 – Dislike Moderately
2 – Dislike Very Much
1 – Dislike Extremely

During the test period, 24-hour surveillance was maintained by nonmedical monitors and the attending nursing staff. The subjects were examined by a physician at least once each day and physiological measurements obtained as indicated in table II to assess their health and the effects, if any, of the fuel cell water.

TABLE II.

PHYSIOLOGICAL MEASUREMENTS PERFORMED ON TEST SUBJECTS

Measurement	*Frequency*
Water intake (volume)	Daily
Food intake (weight, including water)	Daily
Urine output (volume)	Daily
Urine water: solid ratio	Daily
Urination frequency and volume/output	Daily
Defecation frequency	Daily
Gross fecal appearance (consistency, color, odor)	All defecations
Complete urine analysis	Daily
Complete blood count, reticulocyte and platelet count	5 times
Serum glutamic pyruvic transaminase (SGPT)	5 times
Physical examination	Daily
Blood pressure	Daily
Oral temperature	Daily
Pulse rate	Daily
Weight	Daily
Respiration rate	Daily
Blood volume, Radio Iodinated Serum Albumin (RISA)	3 times

Additional experimental conditions were as follows:

1. Bathing, shaving, changing clothes and bed linen were not permitted during the study to simulate the same constraints of space flight. Chemically saturated wipes were furnished for wiping the face and hands after meals and for use on other body areas as needed.

2. All water lines into the ward and rooms, except to the commode, were shut off so that no outside water source was available to the subjects.

3. A daily log was kept by each subject and by the medical monitor concerning the daily status of each subject.

SECTION III.

Results

The data concerning water balance, physiological measurements and organoleptic evaluations are summarized in tables III-X. These tables contain averages of values representing determinations performed at daily or sporadic intervals. The results of the complete urine analyses could not be summarized because of the large number of determinations that were made (13 determinations on 8 samples per day). These analyses included reaction, specific gravity, ketone, qualitative albumin, qualitative sugar, RBC, WBC, blood, epithelium, casts, mucus, bacteria, and crystals. The results were all within clinically accepted normal values.

The total dietary, fuel cell and distilled water intake, and the percentage of each for the 2-week period are summarized in table III. The total water intake ranged from 40 to 80 liters. The dietary water constituted from 14.5 to 27.8% of the total water intake. The fuel cell water constituted 73-76% of the total water intake of the four subjects who drank the water for two weeks. For the two subjects who drank fuel cell water for only one week, it ranged between 36 and 38%.

Table IV shows that the mean daily nondietary water intake for all subjects was 2622 ml, and individual intake ranged from 2082 to 4681 ml. All subjects consumed as a minimum the amount required for the study (2000 ml).

All of the subjects showed consistent and normal urinary outputs and fecal voids. The fecal void patterns were consistent with the subjects' normal void patterns. There was no reported abnormal bowel movement or fecal consistency. The specific gravity and percent solids were consistent with each subject's pattern of water intake and urine output.

TABLE III

DIETARY, FUEL CELL, AND DISTILLED WATER INTAKE

Subject No.	*Total ml*	*Dietary*		*Fuel Cell Water*		*Distilled Water*	
		ml	*%*	*ml*	*%*	*ml*	*%*
1	50,433	11,078	22.0	18,040	35.8	21,315	42.2
2	40,588	11,078	27.3	29,510	72.7	0	0
3	47,238	11,078	23.5	36,160	76.5	0	0
4	40,223	11,078	27.5	29,145	72.5	0	0
5	47,268	11,078	23.4	36,190	76.6	0	0
6	39,848	11,078	27.8	0	0	28,770	72.2
7	76,613	11,078	14.5	29,085	38.0	36,450	47.5
8	40,238	11,078	27.5	0	0	29,160	72.5

TABLE IV.

SUMMARY OF MEAN 24-HOUR WATER INTAKE, URINE AND FECAL VOIDS, SPECIFIC GRAVITY, AND PERCENT SOLIDS

Subject No.	*Water Intake* ml*	*Urine Output ml*	*Average Urine Void ml*	*Number Fecal Voids*	*Specific Gravity*	*% Solids*
1	2811	2740	313	0.6	1.006	1.6
2	2108	2427	435	1.0	1.009	2.0
3	2593	2777	410	0.7	1.006	1.6
4	2082	2259	301	0.9	1.008	1.9
5	2585	2722	377	0.7	1.008	1.8
6	2041	2094	298	1.5	1.009	2.1
7	4681	4978	352	1.3	1.004	0.9
8	2083	2248	297	0.4	1.009	2.0
Mean	2622	2767	348	0.9	1.007	1.7
Range	(2082-4681)	(2094-4978)	(297-435)	(0.4-1.5)	(1.004-1.009)	(0.9-2.1)

*Exclusive of dietary water

The data in table V indicate the insensible water for each subject. It is notable that no one 3-day value is more than 20% from the mean of the four 3-day values. The mean 24-hour insensible water ranges from 517 to 856 ml. All of these values can be considered normal in view of the limited activities of the subjects and the air-conditioned facilities in which they were housed.

Table VI is a summary of the hematological data. The pretest data were derived from a single sample, while the experimental period data are the average of four samples. All the data are in the normal range. The fluctuations between pretest and experimental period data are random and occur randomly among the subjects.

Table VII is a summary of blood chemistry determinations. All the values fall within the normal range. The fluctuations between pretest and experimental periods are random and occur randomly among the subjects.

Table VIII is a summary of Radio Iodinated Serum Albumin (RISA) blood volumes. The mean, standard deviation, and coefficient of variation are presented for three separate blood volume determinations. The coefficient of variation is between 3 and 5% for five subjects and between 7 and 9% for three subjects. The fluctuations in individual blood volumes were random and the fluctuations of the coefficient of variation are randomly distributed. There were no significant changes in blood volume in any of the subjects.

TABLE V.

TOTAL WATER INTAKE AND URINE OUTPUT DIFFERENCE*

ml/3-Day Periods

3-Day Period	*Subject No.*							
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
1	3052	1400	1997	1686	2806	2826	1898	1998
2	2377	1482	2188	1847	1767	1763	1929	2034
3	2669	1601	2109	1894	1973	2289	1194	1701
4	2177	946	2012	1829	2362	2398	1185	1358
Mean, 3 day	2569	1357	2077	1814	2227	2319	1552	1773
Mean, 1 day	856	452	692	605	742	580	517	591

*Numbers in the table are the differences between water intake and urine output and hence indicate insensible water.

TABLE VI.

SUMMARY OF HEMATOLOGICAL DATA*

Subject No.		Erythrocytes /cu mm	Hemoglobin g%	Leukocytes /cu mm	Polymorphonuclear Non-Seg. %	Polymorphonuclear Seg.	Lymphocytes %	Monocytes %	Eosinophils %	Basophils %	Platelets /cu mm	Reticulocytes %
1	Pre	5.34×10^6	15.8	7,725	0	48	36	12	2	0	950,520	1.6
	Exp	5.17×10^6	15.7	5,935	0.75	52	37	7	3	0.25	956,425	1.3
2	Pre	5.43×10^6	15.2	4,493	2	56	32	3	7	0	1,107,720	0.7
	Exp	5.82×10^6	16.1	6,697	0.5	60	31	7	1.3	0.25	919,548	1.6
3	Pre	6.07×10^6	12.8	4,686	0	35	55	6	3	1	588,790	0.7
	Exp	6.17×10^6	13.1	5,234	0.25	44	45	7	3	0.5	893,910	3.2
4	Pre	5.13×10^6	15.0	4,702	1	51	33	8	7	0	564,300	0.9
	Exp	5.27×10^6	15.3	4,982	0	51	39	5	5	0	992,900	1.9
5	Pre	4.56×10^6	14.2	5,950	0	57	35	5	2	1	597,360	1.2
	Exp	5.00×10^6	15.5	6,174	0	57	35	5	3	0	834,258	1.4
6	Pre	5.32×10^6	15.5	5,745	0	40	40	6	14	0	521,360	1.5
	Exp	5.33×10^6	15.1	6,028	0	45	38	7	10	0.75	737,245	2.3
7	Pre	4.96×10^6	14.0	6,859	0	63	31	5	1	0	714,240	1.4
	Exp	5.18×10^6	14.6	5,725	0	51	40	8	2	0.25	1,255,000	1.7
8	Pre	5.54×10^6	16.4	6,896	0	69	30	1	0	0	756,440	1.0
	Exp	5.07×10^6	15.7	7,250	0.25	60	32	7	1	0.25	1,407,085	3.1

*Pretest data from single samples.
Experimental period data average of four samples.

TABLE VII.

SUMMARY OF BLOOD CHEMISTRY*

Subject No.	*Creatinine (mg/100 ml)*	*Urea Nitrogen (mg/100 ml)*	*SGPT (units)†*
1 Pre	1.2	20	3
Exp	1.3	15	8
2 Pre	1.2	13	6
Exp	1.3	13	7
3 Pre	1.4	10	13
Exp	1.3	12	13
4 Pre	1.4	15	13
Exp	1.1	15	19
5 Pre	1.2	13	4
Exp	1.2	18	6
6 Pre	1.3	17	7
Exp	1.4	16	9
7 Pre	1.4	11	7
Exp	1.2	14	14
8 Pre	1.4	15	4
Exp	1.4	13	5

*Pretest data from single samples.
Experimental period data average of four samples.
†Pooled serum standard: 6, 5, 4, 6, 7.

Table IX is a summary of the physiological measurements. The pretest data are derived from a single measurement and the experimental period data are averaged from 14 daily measurements. In general, blood pressure, pulse rate, temperature, respiration, and body weight remained constant throughout the experiment for all subjects.

The clinical summaries of the attending physicians indicated that significant changes in the clinical data did not occur nor were any symptoms observed that were related to the experimental conditions.

The organoleptic ratings for each meal by category, overall meal, water with meal and water without meal were averaged each day. The combined averages are shown in table X. The daily ratings were averaged over the 14-day period, and the mean, standard deviation, and coefficient of variation calculated for the type of water used are presented in the summary. The overall meal ratings ranged from 6 to 8, with only one subject rating the food at 6. Water with meal did not rate higher than water without meal. This is peculiar because most of the subjects agreed during the debriefing that water with the meal was more palatable. Water was rated lower than the food items because very few of the subjects drink water as such. Subject No. 3 was most pointed about

TABLE VIII.

SUMMARY OF RISA BLOOD VOLUMES*

Subject No.	Total Blood Volume		Plasma Volume		Red-Cell Volume	
	Mean SD	Co. Var. %	Mean SD	Co. Var. %	Mean SD	Co. Var. %
1	5505 ± 285	5	2941 ± 147	5	2552 ± 184	7
2	5686 ± 207	4	3093 ± 250	8	2593 ± 57	2
3	5347 ± 145	3	3145 ± 106	3	2202 ± 155	7
4	5305 ± 420	8	2972 ± 196	7	2331 ± 177	8
5	4829 ± 288	6	2631 ± 121	5	2197 ± 190	9
6	5159 ± 289	6	2857 ± 254	9	2302 ± 133	6
7	4642 ± 122	3	2627 ± 97	4	2016 ± 118	6
8	5527 ± 358	6	2956 ± 156	5	2571 ± 207	8

*Radio Iodinated Serum Albumin (RISA).
I^{131} – labelled serum albumin dilution technique.

TABLE IX.

SUMMARY OF PHYSIOLOGICAL MEASUREMENTS*

Subject No.	Blood Pressure Systolic/Diastolic	Oral Temperature	Pulse	Respirations	Body Weight lb
1 Pre	130 / 72	97.6	72	24	154.0
Exp	115 / 73	96.8	67	17	156.0
2 Pre	120 / 60	97.6	80	20	156.5
Exp	112 / 58	97.5	66	18	156.3
3 Pre	152 / 90	96.8	72	20	217.0
Exp	138 / 80	97.3	67	18	215.8
4 Pre	102 / 74	97.0	72	24	128.8
Exp	115 / 64	97.9	66	18	129.5
5 Pre	118 / 60	97.8	72	20	136.3
Exp	111 / 61	97.6	65	17	135.4
6 Pre	110 / 60	97.0	72	20	135.0
Exp	128 / 80	97.7	71	19	134.8
7 Pre	108 / 76	98.0	76	20	142.0
Exp	128 / 80	97.8	72	18	142.9
8 Pre	120 / 62	97.0	76	16	179.8
Exp	121 / 72	97.5	65	18	182.4

*Pretest data period from single sample.
Experimental period data average of 14 samples.

this in his debriefing comments. According to the data several of the subjects obviously did not evaluate food or drink objectively at each meal. With coefficients of variation of 0, 1, 2 or even 4 or 5% it appears that a particular rating was used over and over. Apparently the fuel cell water per se consumed as the only beverage in amounts greater than 2000 ml per day for 14 days was as readily acceptable as distilled water.

TABLE X.

SUMMARY OF ORGANOLEPTIC RATINGS

Subject No.	*Water*	*Overall Meal*		*Water With Meal*		*Water Without Meal*	
		Mean SD	*Co. Var. %*	*Mean SD*	*Co. Var. %*	*Mean SD*	*Co. Var. %*
1	FCW	7.8 ± 0.2	2	5.7 ± 0.7	12	4.7 ± 0.4	8
	DW	7.2 ± 0.5	7	7.0 ± 0.1	1	6.8 ± 0.3	4
2	FCW	7.6 ± 0.4	5	5.6 ± 0.7	12	5.6 ± 1.0	18
3	FCW	6.7 ± 0.8	12	3.3 ± 0.4	12	3.3 ± 0.4	12
4	FCW	7.0 ± 0.1	1	5.6 ± 0.4	7	5.5 ± 0.5	9
5	FCW	6.0 ± 0.4	7	5.0 ± 0.4	8	4.7 ± 0.4	8
6	DW	6.7 ± 0.7	10	4.9 ± 0.2	4	4.9 ± 0.1	2
7	FCW	7.8 ± 0.4	5	6.6 ± 0.8	12	6.6 ± 0.7	11
	DW	7.8 ± 0.3	4	7.0 ± 0	0	7.0 ± 0	0
8	DW	8.2 ± 0.2	2	7.0 ± 0	0	7.0 ± 0.1	1

FCW = fuel cell water
DW = distilled water

SECTION IV.

Discussion

The fuel cell water used in this test was demonstrated to be as acceptable as distilled water in constituting part of a daily diet for at least a 2-week period. Although fuel cell water was detected by some of the subjects because of its slightly acid or sour taste, there was an apparent loss of sensitivity to this sourness with time. Several of the subjects who were smokers adjusted to the nonsmoking situation by drinking water. They seemed to drink a glass of water instead of lighting a cigarette. Even the subjects who drank less water at the beginning of the experiment, eventually increased their water intake during the second week.

The subjects showed no clinical symptoms as a result of the 2-week confinement and test regimen. Hematology, blood chemistry, blood volume, physiological measurements, and urinalysis were all normal. Kidney and bowel movements were regular, and there were no gastrointestinal irregularities. The subjects complained about the hard candy, which caused some oral irritation. Although the hard candy was included in the diet to increase in-between-meal drinking of water, it was apparently unnecessary and was more troublesome than helpful.

The lack of personal hygiene caused little discomfort. The subjects complained only early in the experiment about body odor, but readily adapted to the condition. Dry brushing of the teeth was acceptable and accomplished without complaint. The chemically treated paper wipes were also readily acceptable and were used sparingly.

SECTION V.

Summary and Recommendations

The fuel cell water consumed during the two weeks of this study did not cause any psychological or physiological problems. Organoleptic evaluation indicated that the water was acceptable and the clinical data attested to its lack of effect on the physiological measurements.

With the exception of the low pH which the subjects detected but did not find objectionable and the higher COD and total organic levels, the fuel cell water tested was considered acceptable according to U. S. Public Health Service standards.

Sufficient organic and inorganic contamination occurred in the fuel cell water to permit the growth of significant levels of various bacteria and fungi. Although no enteric or potentially pathogenic microbes were observed, filtration was deemed necessary to improve the water quality to meet the minimum standard of less than 500 viable organisms per milliliter. To decrease microbial and organic contamination of water derived from fuel cells for future use, attempts should be made to minimize entry of contaminants in the water.

Appendix I.

CHEMICAL ANALYSES

The presence of varying amounts of organic and inorganic contaminants in ground water to be used as potable water sources resulted in the establishment of standards by the United States Public Health Service (USPHS). These values are based upon continuous long-term consumption by all members of the population. Potable water for space systems presents different problems because the limited population involved is restricted and the initial sources of water (fuel cell, atmospheric condensate, urine, and waste water) are characterized by different contaminants. The Aerospace Medical Research Laboratories (AMRL) is formulating criteria for space water supplies that are realistic with respect to physiological and mission requirements and for which a monitoring capability may be designed. To insure that the fuel cell water obtained for use in the present study did not present any medical hazard, an extensive analysis was performed to include those criteria established by USPHS. An initial series of determinations was performed by AMRL on nine samples selected at random from the 41 samples received. A more complete analysis was then performed on pooled samples, both filtered and unfiltered (described in appendix II) by the Water Quality Section, Taft Sanitary Engineering Center, USPHS, Cincinnati, Ohio. After pooling and withdrawal of a sample for analysis, the water was filtered and a sample obtained for subsequent analysis. The results of the analyses are shown in table XI. For almost all determinations, the water was considered acceptable by USPHS standards. Only the pH, COD, and total organic carbon exceeded the allowable levels. The low pH of 3 was due to the contamination by sulfonates resulting from breakdown of the polystyrene sulfonate membrane. The recommended allowance* for COD is 150 mg/L; both the filtered and unfiltered samples exceeded this value (270 and 200 mg/L respectively). The total organic carbon level for the unfiltered sample was 40 ppm, less than the 50 ppm set by USPHS. The filtered sample showed a higher value of 89. The higher values obtained with the filtered sample may be attributable to microbial activity, since the filtration procedure was shown to be incomplete. Although deviating from selective criteria, the water was considered nonhazardous for human consumption and the pooled water was made available for the study. This judgment was based primarily on the extensive chemical and microbiological tests that were performed and on the knowledge that the materials in the water that accounted for the deviations were present in nontoxic concentrations.

*Personal Communication with Mr. Robert Kroner, USPHS, Cincinnati, Ohio

TABLE XI.

CHEMICAL ANALYSIS OF FUEL CELL WATER

(Data obtained from AMRL and Taft Sanitary Engineering Center)

Analysis	*Unfiltered*	*Filtered*
ph	2.97	3.03
Conductivity, μmhos/cm	475	255
Total solids, ppm	256	280
Chlorides, ppm	6.0	7.2
Sulfates, ppm	5.0	3.5
Nitrate-N, ppm	—	1.1
Ammonia-N, ppm	1.2	0.8
Phenols, ppm	—	0
Turbidity, Jackson units	5	4
Color, chloroplatinate units	10	25
Odor	slight	none
Taste	none to slight	none to slight
Total organic carbon, ppm	40	89
COD*, ppm	200	270
Total hardness †, ppm	12	8.0
Fluoride, ppm	2.0	2.0
Zinc, ppb	190	35
Cadmium, ppb	<11	<10
Boron, ppb	50	85
Phosphorus, ppb	450	200
Iron, ppb	95	59
Molybdenum, ppb	<6	<5
Manganese, ppb	<3	<3
Aluminum, ppb	170	105
Beryllium, ppb	<0.03	<0.03
Copper, ppb	135	95
Silver, ppb	4.5	<0.5
Nickel, ppb	12	<5
Cobalt, ppb	<6	<5
Lead, ppb	<11	<10
Chromium, ppb	6	<3
Vanadium, ppb	<11	<10
Barium, ppb	18	3
Strontium, ppb	<2	<1
Arsenic, ppb	<70	—
Mercury, ppb	100	—
Titanium, ppb	trace	

*Chemical oxygen demand
†As $CaCO_3$
- - - not performed

Appendix II.

MICROBIOLOGICAL EXAMINATION OF FUEL CELL WATER

To determine the microbiological content of samples of the fuel cell water, selected samples were subjected to routine microbiological analyses. The media used were chosen to enable the isolation of potentially pathogenic species of bacteria. Since the Aerospace Medical Research Laboratories has established a tentative standard of sterility for space system water supplies, the United States Public Health Service standards 1962 were not considered adequate. These standards permit the presence of coliform organisms in a certain percentage of the analyzed samples, depending upon the frequency of analyses and the size of the samples. The standards selected for the fuel cell water studies using human subjects were more stringent in that no pathogens would be permitted, no coliforms would be permitted, and total aerobic count would be no greater than 500/milliliter.

MATERIALS AND METHODS

Media: The following media were used for the detection and enumeration of specific microbes: Phenol Red Broth Base (Difco) plus 1% lactose, for the determination of lactose fermentation; Trypticase Soy Broth (BBL) plus 2% Bacto-Agar for the enumeration of aerobic constituents; Blood Agar (Trypticase Soy Agar plus 5% defibrinated sheep blood), for the detection of hemolytic organisms such as staphylococci and streptococci and other fastidious species; MacConkey Agar (BBL), for the detection of coliform and other enteric bacteria; Mitis-Salvarius Agar (Difco) for the detection of *Streptococcus mitis, St. salvarius,* and enterococci; Salmonello-Shigella (S.S.) Agar (Difco), for the isolation of salmonellae and shigellae; and Sabouraud's Agar, for the isolation of fungi.

Plating Procedure: Nine individual and one pooled sample (a composite of 10-ml aliquots of 32 samples) were diluted individually in Trypticase Soy Broth (0.1 ml in 9.9 ml). Duplicate 0.1-ml aliquots of this dilution and 0.1 ml aliquots of the undiluted samples were placed on the surface of the media used and spread with a sterile glass rod, thus representing 10^{-1} and 10^{-3} dilutions. The plates were incubated at 37C for 48 hours and examined for typical colonies. To determine the presence of the coliform group with larger sample sizes, tubes containing 50 ml of lactose broth were inoculated with 10 ml of the samples and incubated for 48 hours at 37C. Observations were made for acid production and the presence of gas by means of inverted vials placed within the lactose broth tubes.

Additional Trypticase Soy Agar plates were inoculated with the fuel cell water after the experiment had terminated, and incubated at room temperature for 3 days. The 10^{-1} dilution showed many fungal colonies and bacterial colonies of two types that were present in very high numbers. One of the types of bacterial colonies were pinpoint in size and consisted of what appeared to be minute cocci or diplococci surrounded by a capsule-like layer. The cells were too small to discern their gram reactivity. The larger colonies were 0.5 mm in diameter and were composed of gram negative bacilli. Thus it is mandatory that in future analyses of space water supplies the USPHS recommendation of incubating a set of plates at 25C be included. It is further suggested that analyses include the utilization of anaerobic media such as thioglycollate broth to detect the presence of obligate anaerobes. Since some spore formers require heat shock for spore germination, this technique should also be included.

Filtration Procedure: After individual and pooled samples were examined microbiologically and chemically, all 41 samples were pooled in a large plastic container. The pooled water was poured through a Buchner funnel containing Whatman No. 2 filter paper and then forced through a 15.2-cm diameter Millipore® membrane filter of 0.45-micron porosity with the aid of a peristaltic pump. The filtrate was collected in sterile carboys and then transferred to a 286 liter plastic tank that had been cleaned with 70% ethyl alcohol. Approximately 208 liters were obtained.

RESULTS AND DISCUSSION

In table XII are presented the results of the analyses on the selected individual and pooled water samples. Examination of the lactose broth results and the E.M.B. data reveals the tested samples to be free of coliform contamination. None of the 50 fermentation tubes showed the presence of gas in an acid medium, thus virtually eliminating the possibility of the presence of the coliform group. Those colonies that were capable of growth on E.M.B. were definitely not enteric organisms. Preliminary analysis indicated these organisms were primarily pseudomonads. The total aerobic count data show various samples to contain significant numbers of bacteria (and fungi). Since most of the samples were stored for periods of up to 10 months, extremely high number of viable microbes could not be expected since normal population decay would have occurred. Non-lactose fermenting enteric pathogens were not isolated as indicated by the lack of typical growth on MacConkey and Salmonella-Shigella agars. Typical enterococci were not observed on Mitis-Salivarius agar. Those organisms growing on this media were gram negative cocco-baccilli with marked bipolor staining. With the exception of No. 25, all samples contained some degree of fungal contamination. Although fungal counts are not truly indicative of the amount of fungal protoplasm contained in a sample, they may be used as general indices of the degree of fungal contamination.

Thus, the water samples initially tested were free of coliforms, enterococci, and enteric pathogens. However, the bacterial and fungal levels were higher than desired and filtration was performed as described on all the water. The results of analyses on the filtered water, shown in table XIII, indicate the filtration process removed most if not all of the bacterial contaminants and the majority of the fungal contaminants. Since the container used to store the filtered water was not sterilized and the container lid did not insure an airtight closure, contamination from the atmosphere could have occurred. In addition, the membrane filter used was not sterilized and may have served as the source of subsequent contamination. This water, however, was judged to be safe for consumption and was made available to the test subjects.

On the eleventh day of the experiment some particulate matter was observed in the water. A sample was taken after the experiment had terminated and the reservoir was examined. The bottom and part of all four sides of the container were coated with what appeared to be fungal growth. Microscopic observation of material scraped from the container confirmed this observation. Several media were inoculated, most of which showed no growth after incubation. However, the Trypticase Soy Agar plates contained nine colonies on each duplicate representing 90 organisms per milliliter. Fungi were also recovered. The bacteria proved to be gram-negative coccobacilli observed previously. It would thus appear that the filtration procedure may have not been completely successful, permitting very few cells to enter the water reservoir. Their number may have been too few to detect initially and were found only after some increase in numbers had occurred. The low pH of the water may have prevented their growth to higher levels.

The presence of the fungi in significant concentrations, indicative of a favorable environment for the growth of such forms, requires that sufficient precaution be taken with fuel cell water to preclude their presence as well as other kinds of microorganisms.

TABLE XII.

MICROBIOLOGICAL CONTENT OF FUEL CELL WATER SAMPLES

Media		*0*	*5*	*10*	*15*	*20*	*25*	*30*	*35*	*40*	*Pooled*
Lactose Broth	1	A, No G	A, No G	No A, No G	A, No G	No A, No G	SA, No G	No A, No G	No A, No G	SA, No G	A, No G
	2	No A, No G	A, No G	No A, No G	A, No G	No A, No G	SA, No G	No A, No G	No A, No G	SA, No G	A, No G
	3	A, No G	A, No G	No A, No G	A, No G	No A, No G	SA, No G	No A, No G	No A, No G	SA, No G	A, No G
	4	A, No G	A, No G	No A, No G	A, No G	No A, No G	SA, No G	No A, No G	A, No G	SA, No G	A, No G
	5	A, No G	A, No G	No A, No G	A, No G	No A, No G	SA, No G	No A, No G	No A, No G	A, No G	A, No G
Trypticase Soy Agar*		$34X10^3$	10B 55F	NEG	$6X10^3$B $6.5X10^3$F	595	NEG	$40X10^3$B	315B	NEG	$23X10^3$B
EMB Agar**		$29.5X10^3$	180	NEG	$15X10^3$	$17X10^3$	NEG	$60.5X10^3$	170	NEG	$21.5X10^3$
Salmonella-Shigella Agar		NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
MacConkey Agar		NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Mitis-Salivarius Agar		NEG	NEG	NEG	10^2-10^3	NEG	NEG	$17X10^3$	39	NEG	$34X10^3$
Sabouraud Agar		TNTC	$7.5X10^3$	175	$18.5X10^3$	TNTC	NEG	$62X10^3$	$13X10^3$	$7X10^3$	TNTC

*Colony count per ml., B = bacteria, F = fungi for this medium; Sebouraud counts refer to fungal colonies, all others to bacterial colonies.
**Colonies observed were not coliforms.
NEG = No Colonies observed.
A = Acid production, SA = Slight acid production, G = Gas production.
TNTC = Too numerous to count.

TABLE XIII.

MICROBIOLOGICAL CONTENT OF FILTERED FUEL CELL WATER

Sample	*Lactose Broth*	*Trypticase Soy Agar*	*Blood Agar*	*MacConkey Agar*	*Mitis Salivarius Agar*	*Salmonella-Shigella Agar*	*Sabouraud Agar*
Filtered Fuel Cell Water	No A, No G	NEG	NEG	NEG	NEG	NEG	70*
Control (Tap Water)	No A, No G	NEG	NEG	NEG	NEG	NEG	NEG
Control (Distilled Water)	No A, No G	NEG	NEG	NEG	NEG	NEG	NEG

*Colony count per ml
A = Acid production
G = Gas production
NEG = No Colonies observed

Fungal Identification

Microscopic examination was made of colonies of fungi appearing on plates inoculated with fuel cell water. Identifications were made to genus wherever possible, but no attempt was made to identify to species. In many instances, colonies were growing close together or one was overgrowing another and many bacterial colonies were associated with the fungi, thus producing a typical growth and making identification difficult. Since identifiable colonies of *Hormodendrum* were found growing in the water in several of the plastic containers when they were first brought to this laboratory, it was not surprising to find this to be the most common fungus on plates inoculated with this water. Other than this general comment, no further attempt was made to rate the various fungi as to frequency of occurrence.

On plates of Sabouraud's medium which had been inoculated with fuel cell water and then incubated at 25C (room temperature), the following fungi were found:

1. *Hormodendrum* sp.
2. *Aspergillus* sp.
3. *Penicillium* sp.
4. *Sporotrichum* sp.
5. *Cephalosporium* sp.
6. A large yeast (of the *Saccharomyces* type)
7. A small yeast (probably *Hansenula* type)

8, 9, 10. Three nonsporulating members of the Dematiaceae (Fungi Imperfecti, order Moniliales)

11, 12. Two nonsporulating light mycelial types probably of the order Moniliales, Fungi-Imperfecti

From plates inoculated with filtered fuel cell water and incubated at room temperature the following were obtained:

1. *Hormodendrum* sp.
2. *Botrytis* sp.
3. *Paecilomyces* sp.
4. A poorly sporulating synnematous type which is probably *Isaria* sp. (Fungi Imperfecti, order Moniliales, family Stilbaceae)

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13. ABSTRACT

Water obtained from a hydrogen-oxygen fuel cell was subjected to chemical, organoleptic, and microbiological analyses and found to be acceptable according to the U. S. Public Health Service Standards. To ascertain the acceptability of this water, eight male subjects were confined to a self-care unit at Miami Valley Hospital, Dayton, Ohio, for a 2-week period and served a controlled 4-meal-per-day diet, low in water content. A minimum of 2 liters per day of either distilled water or fuel cell water was drunk by the subjects as follows: two subjects were served distilled water for 2 weeks, serving as controls; four were served fuel cell water for 2 weeks; and two were served fuel cell water for one week, followed by distilled water for the second week. Twenty-four hour surveillance of the subjects was maintained. They were examined by a physician at least once daily; daily urinary and periodic blood samples were taken for routine laboratory examination. The subjects showed no clinical symptoms at the end of the test period, and the fuel cell water was found to be as acceptable as distilled water in constituting part of a daily diet for the 2-week period.

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